



## SEQUENCE LISTING

<110> Cox III, George N.  
Case, Casey Christopher  
Eisenberg, Stephen P.  
Jarvis, Eric E.  
Spratt, Sharon K.

<120> REGULATION OF ENDOGENOUS GENE EXPRESSION IN CELLS  
USING ZINC FINGER PROTEINS

<130> 8325-0002.10 / S2-US3

<140> 09/706,243

<141> 2000-11-03

<160> 43

<170> PatentIn Ver. 2.0

<210> 1

<211> 25

<212> PRT

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence:exemplary motif  
of C2H2 class of zinc finger proteins (ZFP)

<220>

<221> MOD\_RES

<222> (2)..(3)

<223> Xaa = any amino acid

<220>

<221> MOD\_RES

<222> (4)..(5)

<223> Xaa = any amino acid, may be present or absent

<220>

<221> MOD\_RES

<222> (7)..(18)

<223> Xaa = any amino acid

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<221> MOD\_RES

<222> (20)..(22)

<223> Xaa = any amino acid

<220>

<221> MOD\_RES

<222> (23)..(24)

<223> Xaa = any amino acid, may be present or absent

<400> 1

Cys Xaa Xaa Xaa Xaa Cys Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa

1

5

10

15

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TC 1700

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APR 26 2002

TECH CENTER 1600/2900

Xaa Xaa His Xaa Xaa Xaa Xaa Xaa His  
20 25

<210> 2

<211> 10

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence:ZFP target site  
with two overlapping D-able subsites

<220>

<221> modified\_base

<222> (1)..(2)

<223> n = g,a,c or t

<220>

<221> modified\_base

<222> (5)

<223> n = g,a,c or t

<220>

<221> modified\_base

<222> (8)

<223> n = g,a,c or t

<220>

<221> modified\_base

<222> (9)

<223> n = a,c or t; if g, then position 10 cannot be g  
or t

<220>

<221> modified\_base

<222> (10)

<223> n = a or c; if g or t, then position 9 cannot be g

<400> 2

nngkngknnn

10

<210> 3

<211> 10

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence:ZFP target site  
with three overlapping D-able subsites

<220>

<221> modified\_base

<222> (1)..(2)

<223> n = g,a,c or t

<220>

<221> modified\_base

<222> (5)

<223> n = g,a,c or t

<220>

<221> modified\_base

<222> (8)

<223> n = g,a,c or t

<400> 3

nngkngkngk

10

<210> 4

<211> 5

<212> PRT

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence:linker

<400> 4

Asp Gly Gly Gly Ser

1

5

<210> 5

<211> 5

<212> PRT

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence:linker

<400> 5

Thr Gly Glu Lys Pro

1

5

<210> 6

<211> 9

<212> PRT

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence:linker

<400> 6

Leu Arg Gln Lys Asp Gly Glu Arg Pro

1

5

<210> 7

<211> 4

<212> PRT

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence:linker

<400> 7  
Gly Gly Arg Arg  
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<210> 8  
<211> 5  
<212> PRT  
<213> Artificial Sequence

<220>  
<223> Description of Artificial Sequence:linker

<400> 8  
Gly Gly Gly Gly Ser  
1 5

<210> 9  
<211> 8  
<212> PRT  
<213> Artificial Sequence

<220>  
<223> Description of Artificial Sequence:linker

<400> 9  
Gly Gly Arg Arg Gly Gly Gly Ser  
1 5

<210> 10  
<211> 9  
<212> PRT  
<213> Artificial Sequence

<220>  
<223> Description of Artificial Sequence:linker

<400> 10  
Leu Arg Gln Arg Asp Gly Glu Arg Pro  
1 5

<210> 11  
<211> 12  
<212> PRT  
<213> Artificial Sequence

<220>  
<223> Description of Artificial Sequence:linker

<400> 11  
Leu Arg Gln Lys Asp Gly Gly Gly Ser Glu Arg Pro  
1 5 10

<210> 12

<211> 16  
<212> PRT  
<213> Artificial Sequence

<220>  
<223> Description of Artificial Sequence:linker

<400> 12  
Leu Arg Gln Lys Asp Gly Gly Gly Ser Gly Gly Gly Ser Glu Arg Pro  
1 5 10 15

<210> 13  
<211> 25  
<212> DNA  
<213> Artificial Sequence

<220>  
<223> Description of Artificial Sequence:ZFP target site  
region surrounding initiation site of vascular  
endothelial growth factor (VEGF) gene containing  
two 9-base pair target sites

<220>  
<221> protein\_bind  
<222> (4)..(12)  
<223> upstream 9-base pair ZFP VEGF1 target site

<220>  
<221> protein\_bind  
<222> (14)..(22)  
<223> downstream 9-base pair ZFP VEGF3a target site

<400> 13  
agcggggagg atcgcgagg cttgg 25

<210> 14  
<211> 298  
<212> DNA  
<213> Artificial Sequence

<220>  
<223> Description of Artificial Sequence:VEGF1 ZFP  
construct targeting upstream 9-base pair target  
site in VEGF promoter

<220>  
<221> CDS  
<222> (2)..(298)  
<223> VEGF1

<400> 14  
g gta ccc ata cct ggc aag aag aag cag cac atc tgc cac atc cag ggc 49  
Val Pro Ile Pro Gly Lys Lys Lys Gln His Ile Cys His Ile Gln Gly  
1 5 10 15

tgt ggt aaa gtt tac ggc aca acc tca aat ctg cgt cgt cac ctg cgc	97
Cys Gly Lys Val Tyr Gly Thr Thr Ser Asn Leu Arg Arg His Leu Arg	
20 25 30	
tgg cac acc ggc gag agg cct ttc atg tgt acc tgg tcc tac tgt ggt	145
Trp His Thr Gly Glu Arg Pro Phe Met Cys Thr Trp Ser Tyr Cys Gly	
35 40 45	
aaa cgc ttc acc cgt tgc tca aac ctg cag cgt cac aag cgt acc cac	193
Lys Arg Phe Thr Arg Ser Ser Asn Leu Gln Arg His Lys Arg Thr His	
50 55 60	
acc ggt gag aag aaa ttt gct tgc ccg gag tgt ccg aag cgc ttc atg	241
Thr Gly Glu Lys Lys Phe Ala Cys Pro Glu Cys Pro Lys Arg Phe Met	
65 70 75 80	
cgt agt gac cac ctg tcc cgt cac atc aag acc cac cag aat aag aag	289
Arg Ser Asp His Leu Ser Arg His Ile Lys Thr His Gln Asn Lys Lys	
85 90 95	
ggt gga tcc	298
Gly Gly Ser	

<210> 15

<211> 99

<212> PRT

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence:VEGF1 ZFP  
construct targeting upstream 9-base pair target  
site in VEGF promoter

<400> 15

Val Pro Ile Pro Gly Lys Lys Lys Gln His Ile Cys His Ile Gln Gly
1 5 10 15

Cys Gly Lys Val Tyr Gly Thr Thr Ser Asn Leu Arg Arg His Leu Arg
20 25 30

Trp His Thr Gly Glu Arg Pro Phe Met Cys Thr Trp Ser Tyr Cys Gly
35 40 45

Lys Arg Phe Thr Arg Ser Ser Asn Leu Gln Arg His Lys Arg Thr His
50 55 60

Thr Gly Glu Lys Lys Phe Ala Cys Pro Glu Cys Pro Lys Arg Phe Met
65 70 75 80

Arg Ser Asp His Leu Ser Arg His Ile Lys Thr His Gln Asn Lys Lys
85 90 95

Gly Gly Ser

<210> 16

<211> 298

<212> DNA  
<213> Artificial Sequence

<220>  
<223> Description of Artificial Sequence:VEGF3a ZFP  
construct targeting downstream 9-base pair target  
site in VEGF promoter

<220>  
<221> CDS  
<222> (2)..(298)  
<223> VEGF3a

<400> 16  
g gta ccc ata cct ggc aag aag aag cag cac atc tgc cac atc cag ggc 49  
Val Pro Ile Pro Gly Lys Lys Lys Gln His Ile Cys His Ile Gln Gly  
1 5 10 15  
tgt ggt aaa gtt tac ggc cag tcc tcc gac ctg cag cgt cac ctg cgc 97  
Cys Gly Lys Val Tyr Gly Gln Ser Ser Asp Leu Gln Arg His Leu Arg  
20 25 30  
tgg cac acc ggc gag agg cct ttc atg tgt acc tgg tcc tac tgt ggt 145  
Trp His Thr Gly Glu Arg Pro Phe Met Cys Thr Trp Ser Tyr Cys Gly  
35 40 45  
aaa cgc ttc acc cgt tcg tca aac cta cag agg cac aag cgt aca cac 193  
Lys Arg Phe Thr Arg Ser Ser Asn Leu Gln Arg His Lys Arg Thr His  
50 55 60  
acc ggt gag aag aaa ttt gct tgc ccg gag tgt ccg aag cgc ttc atg 241  
Thr Gly Glu Lys Lys Phe Ala Cys Pro Glu Cys Pro Lys Arg Phe Met  
65 70 75 80  
cga agt gac gag ctg tca cga cat atc aag acc cac cag aac aag aag 289  
Arg Ser Asp Glu Leu Ser Arg His Ile Lys Thr His Gln Asn Lys Lys  
85 90 95  
ggt gga tcc 298  
Gly Gly Ser

<210> 17  
<211> 99  
<212> PRT  
<213> Artificial Sequence

<220>  
<223> Description of Artificial Sequence:VEGF3a ZFP  
construct targeting downstream 9-base pair target  
site in VEGF promoter

<400> 17  
Val Pro Ile Pro Gly Lys Lys Lys Gln His Ile Cys His Ile Gln Gly  
1 5 10 15  
Cys Gly Lys Val Tyr Gly Gln Ser Ser Asp Leu Gln Arg His Leu Arg

20                      25                      30  
 Trp His Thr Gly Glu Arg Pro Phe Met Cys Thr Trp Ser Tyr Cys Gly  
                     35                      40                      45  
 Lys Arg Phe Thr Arg Ser Ser Asn Leu Gln Arg His Lys Arg Thr His  
                     50                      55                      60  
 Thr Gly Glu Lys Lys Phe Ala Cys Pro Glu Cys Pro Lys Arg Phe Met  
                     65                      70                      75                      80  
 Arg Ser Asp Glu Leu Ser Arg His Ile Lys Thr His Gln Asn Lys Lys  
                     85                      90                      95

Gly Gly Ser

<210> 18  
 <211> 29  
 <212> DNA  
 <213> Artificial Sequence

<220>  
 <223> Description of Artificial Sequence:VEGF DNA target  
           site 1 recognition (top) strand

<220>  
 <221> protein\_bind  
 <222> (11)..(19)  
 <223> VEGF DNA ZFP target site 1

<400> 18  
 catgcatagc ggggaggatc gccatcgat 29

<210> 19  
 <211> 29  
 <212> DNA  
 <213> Artificial Sequence

<220>  
 <223> Description of Artificial Sequence:VEGF DNA site 1  
           complementary (bottom) strand

<400> 19  
 atcgatggcg atcctccccg ctatgcatg 29

<210> 20  
 <211> 29  
 <212> DNA  
 <213> Artificial Sequence

<220>  
 <223> Description of Artificial Sequence:VEGF DNA  
           target site 3 recognition (top) strand

<220>



<221> protein\_bind  
 <222> (11)..(19)  
 <223> VEGF DNA ZFP target site 3  
  
 <400> 20  
 catgcatatc gcggaggctt ggcatcgat 29  
  
 <210> 21  
 <211> 29  
 <212> DNA  
 <213> Artificial Sequence  
  
 <220>  
 <223> Description of Artificial Sequence:VEGF DNA target  
 site 3 complementary (bottom) strand  
  
 <400> 21  
 atcgatgcca agcctccgcg atatgcatg 29  
  
 <210> 22  
 <211> 29  
 <212> DNA  
 <213> Artificial Sequence  
  
 <220>  
 <223> Description of Artificial Sequence:primer SPE7  
  
 <400> 22  
 gagcagaatt cggcaagaag aagcagcac 29  
  
 <210> 23  
 <211> 26  
 <212> DNA  
 <213> Artificial Sequence  
  
 <220>  
 <223> Description of Artificial Sequence:primer SPEamp12  
  
 <400> 23  
 gtggtctaga cagctcgta cttcgc 26  
  
 <210> 24  
 <211> 28  
 <212> DNA  
 <213> Artificial Sequence  
  
 <220>  
 <223> Description of Artificial Sequence:primer SPE  
 ampl3  
  
 <400> 24  
 ggagccaagg ctgtggtaaa gtttacgg 28  
  
 <210> 25

<211> 26  
 <212> DNA  
 <213> Artificial Sequence  
  
 <220>  
 <223> Description of Artificial Sequence:primer SPEamp11  
  
 <400> 25  
 ggagaagctt ggatcctcat tatccc 26  
  
  
 <210> 26  
 <211> 83  
 <212> DNA  
 <213> Artificial Sequence  
  
 <220>  
 <223> Description of Artificial Sequence:sequence  
 ligated between XbaI and StyI sites  
  
 <400> 26  
 tctagacaca tcaaaaccca ccagaacaag aaagacggcg gtggcagcgg caaaaagaaa 60  
 cagcacatat gtcacatcca agg 83  
  
  
 <210> 27  
 <211> 39  
 <212> DNA  
 <213> Artificial Sequence  
  
 <220>  
 <223> Description of Artificial Sequence:primer GB19  
  
 <400> 27  
 gccatgccgg tacccatacc tggcaagaag aagcagcac 39  
  
  
 <210> 28  
 <211> 33  
 <212> DNA  
 <213> Artificial Sequence  
  
 <220>  
 <223> Description of Artificial Sequence:primer GB10  
  
 <400> 28  
 cagatcggat ccacccttct tattctggtg ggt 33  
  
  
 <210> 29  
 <211> 589  
 <212> DNA  
 <213> Artificial Sequence  
  
 <220>  
 <223> Description of Artificial Sequence:designed

6-finger ZFP VEGF3a/1 from KpnI to BamHI

<220>

<221> CDS

<222> (2)..(589)

<223> VEGF3a/1

<400> 29

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g gta ccc ata cct ggc aag aag aag cag cac atc tgc cac atc cag ggc 49
  Val Pro Ile Pro Gly Lys Lys Lys Gln His Ile Cys His Ile Gln Gly
    1             5             10             15

tgt ggt aaa gtt tac ggc cag tcc tcc gac ctg cag cgt cac ctg cgc 97
Cys Gly Lys Val Tyr Gly Gln Ser Ser Asp Leu Gln Arg His Leu Arg
          20             25             30

tgg cac acc ggc gag agg cct ttc atg tgt acc tgg tcc tac tgt ggt 145
Trp His Thr Gly Glu Arg Pro Phe Met Cys Thr Trp Ser Tyr Cys Gly
          35             40             45

aaa cgc ttc aca cgt tcg tca aac cta cag agg cac aag cgt aca cac 193
Lys Arg Phe Thr Arg Ser Ser Asn Leu Gln Arg His Lys Arg Thr His
          50             55             60

aca ggt gag aag aaa ttt gct tgc ccg gag tgt ccg aag cgc ttc atg 241
Thr Gly Glu Lys Lys Phe Ala Cys Pro Glu Cys Pro Lys Arg Phe Met
          65             70             75             80

cga agt gac gag ctg tct aga cac atc aaa acc cac cag aac aag aaa 289
Arg Ser Asp Glu Leu Ser Arg His Ile Lys Thr His Gln Asn Lys Lys
          85             90             95

gac ggc ggt ggc agc ggc aaa aag aaa cag cac ata tgt cac atc caa 337
Asp Gly Gly Gly Ser Gly Lys Lys Lys Gln His Ile Cys His Ile Gln
          100            105            110

ggc tgt ggt aaa gtt tac ggc aca acc tca aat ctg cgt cgt cac ctg 385
Gly Cys Gly Lys Val Tyr Gly Thr Thr Ser Asn Leu Arg Arg His Leu
          115            120            125

cgc tgg cac acc ggc gag agg cct ttc atg tgt acc tgg tcc tac tgt 433
Arg Trp His Thr Gly Glu Arg Pro Phe Met Cys Thr Trp Ser Tyr Cys
          130            135            140

ggt aaa cgc ttc acc cgt tcg tca aac ctg cag cgt cac aag cgt acc 481
Gly Lys Arg Phe Thr Arg Ser Ser Asn Leu Gln Arg His Lys Arg Thr
          145            150            155            160

cac acc ggt gag aag aaa ttt gct tgc ccg gag tgt ccg aag cgc ttc 529
His Thr Gly Glu Lys Lys Phe Ala Cys Pro Glu Cys Pro Lys Arg Phe
          165            170            175

atg cgt agt gac cac ctg tcc cgt cac atc aag acc cac cag aat aag 577
Met Arg Ser Asp His Leu Ser Arg His Ile Lys Thr His Gln Asn Lys
          180            185            190

aag ggt gga tcc 589
Lys Gly Gly Ser

```

<223> Description of Artificial Sequence:designed 6-finger ZFP VEGF3a/1 from KpnI to BamHI

[illegible]

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<210> 31
<211> 42
<212> DNA
<213> Artificial Sequence
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<223> Description of Artificial Sequence:JVF9 VEGF3a/1  
target oligonucleotide

<400> 31  
agcgagcggg gaggatcgcg gaggcttggg gcagccgggt ag 42

<210> 32  
<211> 42  
<212> DNA  
<213> Artificial Sequence

<220>  
<223> Description of Artificial Sequence:JVF10 VEGF3a/1  
target oligonucleotide complementary sequence

<400> 32  
cgctctaccc ggctgccccca agcctccgcg atcctccccg ct 42

<210> 33  
<211> 25  
<212> DNA  
<213> Artificial Sequence

<220>  
<223> Description of Artificial Sequence:primer JVF24

<400> 33  
cgcggatccg cccccccgac cgatg 25

<210> 34  
<211> 62  
<212> DNA  
<213> Artificial Sequence

<220>  
<223> Description of Artificial Sequence:downstream  
primer JVF25

<400> 34  
ccgcaagctt acttgatc gtcgtccttg tagtcgtgc cccaccgta ctcgtcaatt 60  
cc 62

<210> 35  
<211> 7  
<212> PRT  
<213> Simian virus 40

<220>  
<221> PEPTIDE  
<222> (1)..(7)  
<223> SV40 large T antigen nuclear localization sequence  
(NLS)

<400> 35  
Pro Lys Lys Lys Arg Lys Val

1

5

<210> 36  
 <211> 61  
 <212> DNA  
 <213> Artificial Sequence

<220>  
 <223> Description of Artificial Sequence:segment from  
 EcoRI to KpnI containing Kozak sequence including  
 initiation codon and SV40 NLS

<400> 36  
 gaattcgcta gcgccaccat ggcccccaag aagaagagga aggtgggaat ccatggggta 60  
 c 61

<210> 37  
 <211> 187  
 <212> DNA  
 <213> Artificial Sequence

<220>  
 <223> Description of Artificial Sequence:segment from  
 KpnI to XhoI containing BamHI site, KRAB-A box  
 from KOX1, FLAG epitope and HindIII site

<400> 37  
 ggtacccggg gatcccgac actggtgacc ttcaaggatg tatttggtga cttcaccagg 60  
 gaggagtgga agctgctgga cactgctcag cagatcgtgt acagaaatgt gatgctggag 120  
 aactataaga acctgggttc cttgggcagc gactacaagg acgacgatga caagtaagct 180  
 tctcgag 187

<210> 38  
 <211> 277  
 <212> DNA  
 <213> Artificial Sequence

<220>  
 <223> Description of Artificial Sequence:inserted  
 fragment from BamHI to HindIII sites

<400> 38  
 ggatccgccc ccccgaccga tgtcagcctg ggggacgagc tccacttaga cggcgaggac 60  
 gtggcgatgg cgcatgccga cgcgctagac gatttcgatc tggacatgtt gggggacggg 120  
 gattccccgg ggccgggatt tccccccac gactccgccc cctacggcgc tctggatatg 180  
 gccgacttcg agtttgagca gatgtttacc gatgcccttg gaattgacga gtacgggtggg 240  
 ggcagcgact acaaggacga cgatgacaag taagctt 277

<210> 39  
<211> 118  
<212> DNA  
<213> Artificial Sequence

<220>  
<223> Description of Artificial Sequence:sequence  
replacing NLS-KRAB-FLAG with NLS-FLAG only

<400> 39  
gaattcgcta gcgccacccat ggcccccaag aagaagagga aggtgggaat ccatggggta 60  
cccggggatg gatccggcag cgactacaag gacgacgatg acaagtaagc ttctcgag 118

<210> 40  
<211> 204  
<212> DNA  
<213> Artificial Sequence

<220>  
<223> Description of Artificial Sequence:insert into  
MluI/BglII sites of pGL3-Control to create  
pVFR1-4x

<400> 40  
acgcgtaagc ttgctagcga gcggggagga tcgaggaggc ttggggcagc cgggtagagc 60  
gagcggggag gatcgaggag gcttggggca gccgggtaga gcgagcgggg aggatcgagg 120  
aggcttgggg cagccgggta gagcgagcgg ggaggatcgc ggaggcttgg ggcagccggg 180  
tagagcgctc agaagcttag atct 204

<210> 41  
<211> 4  
<212> DNA  
<213> Artificial Sequence

<220>  
<223> Description of Artificial Sequence: "D-able" site  
motif

<400> 41  
nngk

4

<210> 42  
<211> 4  
<212> DNA  
<213> Artificial Sequence

<220>  
<223> Description of Artificial Sequence: D-able site  
subtype

<400> 42

nngg

4

<210> 43

<211> 4

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: D-able site  
subtype

<400> 43

nngt

4

D)  
Cont